Vesicle dynamics in elongation flow: Wrinkling instability and bud formation.

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We present experimental results on the relaxation dynamics of vesicles subjected to a time-dependent elongation flow. We observed and characterized a new instability, which results in the formation of higher order modes of the vesicle shape (wrinkles), after a switch in the direction of the gradient of the velocity. This surprising generation of membrane wrinkles can be explained by the appearance of a negative surface tension during the vesicle deflation, due to compression in a sign-switching transient. Moreover, the formation of buds in the vesicle membrane has been observed in the vicinity of the dynamical transition point.

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Giant unilamellar vesicles (GUV's), formed by a closed phospholipid bilayer, appear to be a well-defined simplified system for studying physical aspects of the dynamics of biological cells. Equilibrium mechanical properties of vesicles are relatively well understood. Vesicles exhibit a variety of equilibrium shapes that correspond to minima of the membrane Helfrich's energy [1]. The non-equilibrium dynamics of a vesicle subjected to an external flow received intensive attention in numerous theoretical [2, 3, 4, 5, 6], numerical [7, 8, 9, 10], and experimental [11, 12, 13, 14, 15, 16] studies. In simple shear flows a vesicle exhibits several types of motion – tank treading, tumbling and trembling [13, 14] (also called vacillating-breathing [4] or swinging [10]), depending on its location in the space of the system control parameters (viscosity contrast, excess area, shear rate) [6, 10, 14].

There is, however, a lack of experimental observations in the regime of transient dynamics, when the system undergoes a non-equilibrium relaxation toward one of its dynamically stable states. In stationary shear flows only the lowest order modes (usually just the second order modes) characterize the various dynamical states of the vesicles observed, since the energy contribution from higher order excitation modes, if present, would be much higher. However, as pointed out recently [17], the flow may happen to impose a negative tension on the membrane, which leads to the growth of the higher order modes and to a shape instability.

Let us consider the Helfrich free energy functional in a general form [1]:

$$F = \int dA \left[\frac{\kappa}{2}h^2 + \sigma\right], \qquad (1)$$

where κ is the bending rigidity of the membrane, h is the local curvature and σ is the vesicle surface tension, which is the Lagrange multiplier corresponding to the surface area conservation constraint. If we consider for simplicity just a flat membrane, which can be parameterized by a height function $u(x, y)$, then the expansion of the functional F in the Fourier space up to second order in u gives: $F^{(2)} = \frac{1}{2}\sum_k (\kappa k^4 + \sigma k^2)|u_k|^2$. One notices that modes u_k with $k < \sqrt{|\sigma|/\kappa}$ become unstable for $\sigma < 0$, resulting in the generation of the higher-order modes becoming energetically more favorable. One possibility to experimentally realize a negative surface tension of the vesicle membrane is the use of a time dependent flow, where the sign of the velocity gradients undergoes a fast change, under which a vesicle becomes temporarily deflated. The simplest realization of this idea is a plane elongation (hyperbolic) flow: $v_x = \dot{\epsilon}x, \quad v_y = -\dot{\epsilon}y, \quad v_z = 0$.

In this letter, we present the first experimental study of giant vesicle dynamics in such time-dependent, transient plane hyperbolic flow. We study the vesicle relaxation towards a new stationary state in two cases: from an equilibrium state when the elongation flow is suddenly turned on, $\dot{\epsilon}(t) = H(t)\dot{\epsilon}_0$, where $H(t)$ is the Heaviside step function, and when the elongation flow is suddenly reversed, $\dot{\epsilon}(t) = sign(t)\dot{\epsilon}_0$, i.e. v_{xx} changes from $-\dot{\epsilon}_0$ to $\dot{\epsilon}_0$. The stationary, stretched state is known to obey $D_{sat} = \sqrt{15\Delta/32\pi}$, with $\phi = \{0; \pi/2\}$ [2, 5, 17]. Here $D = (L-B)/(L+B)$, L and B are the large and small semi-axis of the elliptical approximation of the vesicle cross-section and ϕ is the inclination angle with respect to the x axis. We assume that the membrane is impermeable for the time scale of the experiment, with the excess area $\Delta = S/R^2 - 4\pi$, where S is the total surface area of the vesicle and R its effective radius, defined via the volume $V = \frac{4}{3}\pi R^3$.

Measurements of the vesicle dynamics were conducted in the vicinity of the stagnation point ($v_x = v_y = v_z = 0$) via epi-fluorescent or phase contrast microscopy. The flow was produced in a cross-slot micro-channel of 500 μm wide and 320 μm in height manufactured in elastomer (PDMS) by soft lithography [18]. The details of the design and of the arrangement will be published elsewhere. Particle tracking velocimetry measurements of the flow field show that the deviation of the elongation rate $(\Delta\dot{\epsilon})_{xy}/\dot{\epsilon}$ across the size of the observation window is $< 5\%$, deviations of $\dot{\epsilon}$ in the z-direction on the scale of the vesicle were $(\Delta\dot{\epsilon})_z/\dot{\epsilon} < 5\%$, and that the ratio of shear velocity gradient $\dot{\gamma}_z$ to $\dot{\epsilon}$ on the size of the vesicle did not exceed $(\Delta\dot{\epsilon})_z/\dot{\epsilon}$. Experiments were performed in

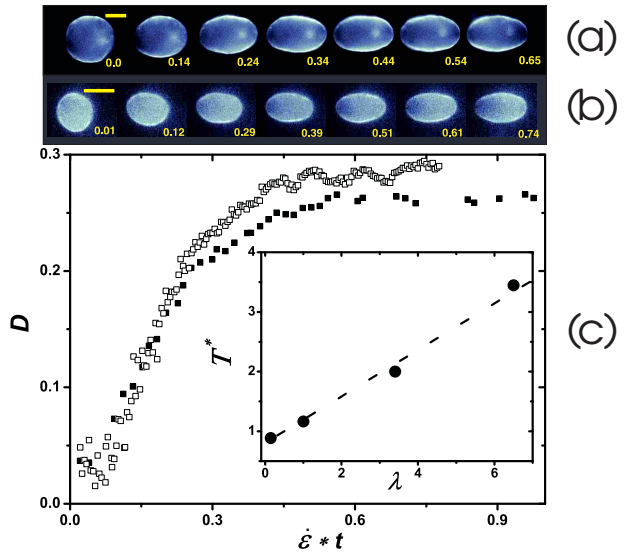

FIG. 1: Relaxation dynamics in elongation flow suddenly turned on. Snapshots of a vesicle with (a) $\lambda = 1$, $\chi = 2.2$, $\Delta = 0.5$, $\dot{\epsilon} = 0.1$, (b) $\lambda = 1$, $\chi = 4.4$, $\Delta = 0.6$, $\dot{\epsilon} = 0.9$. Numbers on the snapshots are the non-dimensional times $t\dot{\epsilon}$, the scale bar is 10 μm. (c) D versus $t\dot{\epsilon}$ with filled squares from data in (a), open squares from data in (b). Inset: statistical average of T^* versus λ.

the range of velocity gradients $\dot{\epsilon} = 0.05 \div 10$. We define the dimensionless strain $\chi = \dot{\epsilon}\eta_{out}R^3/\kappa$, where $\kappa \simeq 10^{-12}$ erg for DOPC [19]. The viscosity of the fluid inside the vesicle, η_{in}, can be different from the viscosity η_{out} of the surrounding fluid, and their ratio λ was varied across the experiments. The lipid solutions consisted of 85% DOPC (Sigma) and 15% NBD-PC (fluorescent lipid, Molecular Probes) dissolved in 9:1 v/v chloroform-methanol solvent (1.8 mg total lipids/ml), or DOPC in the solvent (1.5 mg/ml). The methods and conditions of the preparation of the vesicles for the experiments have been described previously [13, 14, 16].

The first set of the experiments was performed suddenly switching on the flow, starting after the vesicle had relaxed into an equilibrium shape. An initial growth of D, monotonic in time till saturation to $D = D_{sat}$ was observed, whereas ϕ reached the stationary value either of 0 or $\pi/2$ (see Fig.1). We characterize the process for $D << D_{sat}$ by the linear growth time $T^* = \dot{\epsilon}\left[\frac{\Delta D}{\Delta t}\right]^{-1}$. It was found that $T^*(\chi) \approx const$ in the range of $\chi = 1 \div 15$ for a given λ. The dependence of T^* on the viscosity contrast is found to be linear in the range $\lambda = 0.1 \div 7$ (see inset Fig.1c). Averaging is done on the data sets of 30 to 100 points for each λ. These experimental results are consistent with the recent theoretical predictions for the same relaxation to a stationary state for $\chi > 1$. The theory too shows that the relaxation time scales linearly with $\dot{\epsilon}$ and with λ [5, 6, 17].

In the second set of experiments, in which the flow was suddenly switched from $v_{xx} = -\dot{\epsilon}_0$ to $v_{xx} = \dot{\epsilon}_0$ ("suddenly" essentially means that the switching time is much smaller than $\chi/\dot{\epsilon}_0$), vesicles undergo a relaxation from one stretched stationary state ($D = D_{sat}; \phi = \pi/2$) to another one ($D = D_{sat}; \phi = 0$). Surprisingly, we found that above some value of $\chi > \chi_c$ the vesicles develop small wavelength perturbations in the shape, which we call wrinkles (see Fig.2a,b,c).

Quantitative evaluation of the higher order modes of the membrane shape was performed in the following way: the vesicle contour is fitted by an ellipse and the amplitude $A(\theta, \dot{\epsilon}_0 t)$ of the deviation from the elliptical fit is taken as function of the angle θ for every instant of time (see upper inset of Fig.3). Examples of $A(\theta, \dot{\epsilon}_0 t)$ are shown in Fig.2d. The instantaneous Fourier transforms of the amplitudes with respect to θ define the dynamics of the spectrum $u_k(t)$. The temporal evolution of these power spectra is shown in Fig.2e. A difference in the development of the higher k modes for different values of χ can be clearly seen: during the transition from one steady state to another, more higher order modes are excited for larger χ. The evolution of D is shown in Fig.2f in order to precisely define the time interval where the transitional dynamics takes place.

The dependence of the average in time power spectrum of the relaxing modes, $P_k \equiv |u_k|^2$, for different values of χ, is shown in the lower inset of Fig.3. We found that the spectra show a $P_k \propto k^{-4}$ dependence for χ less than some critical value χ_c, that is a well-known spectral decay due to thermal noise [1]. For larger χ, the spectra become rather flat at smaller k, while the higher modes still comply with the equilibrium spectral decay. From the rather sharp transition from the flat to the k^{-4} spectrum around some $k = k_{thr}$, we can postulate that for $\chi > \chi_c$ modes with $k < k_{thr}$ are excited dynamically, while the higher modes are excited not by the flow but rather by thermal noise. We determine χ_c as the threshold above which the modes with $k \geq 3$ are excited ($k = 3$ is the first mode higher than elliptical). To this extent, we define $k^* = \sqrt{\sum_{k=3}^{19} k^2 P_k / \sum_{k=3}^{19} P_k}$. The restriction to $k \leq 19$ is dictated by the image resolution of the smallest vesicles in the experiments. The dependence of k^* on χ, averaged over ≈ 200 data points, is shown in Fig.3. For $\chi < \chi_c$, k^* remains constant, which means that the spectrum of all modes with $k \geq 3$ obeys the equilibrium distribution, k^{-4}. The growth of k^* starts at $\chi = \chi_c$ with $\chi_c = 6.5 \pm 0.8$, which is identified as the onset of the wrinkling instability. The dependence of k^* above the instability threshold can be fitted by $\sim \chi^{1/4}$ in a good agreement with numerical simulations in the same range of χ [17]. All the experiments were done for $\lambda = 1$, and no investigation on the influence of viscosity contrast on the wrinkling phenomena was done.

As we pointed out in the introduction, the generation of higher order modes in the vesicle shape, i.e. wrinkles, observed during the relaxation dynamics should lead to a tremendous increase in the elastic energy, which is unlikely, for a vesicle with positive surface tension. On the other hand, a recent theory predicts that a sudden switch

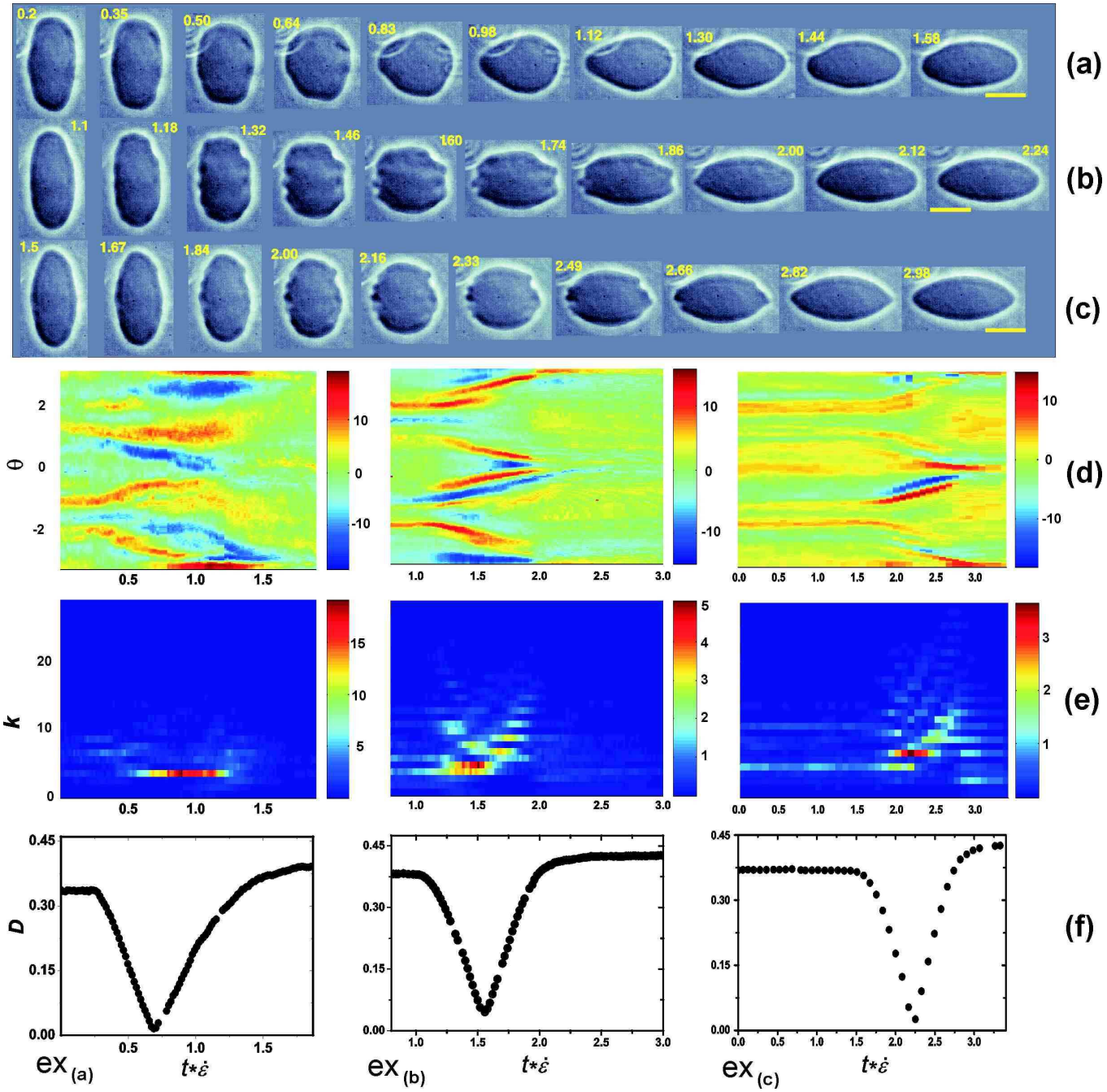

FIG. 2: Wrinkling instability. Snapshots of vesicle dynamics in time-dependent elongation flow at $\lambda = 1$, $\Delta \approx 1$ and: (a) $\chi = 8.1$, (b) $\chi = 81$, (c) $\chi = 323.5$. The scale bar is 20 μm, numbers are $t\dot{\epsilon}$. Plots below the images are the data analysis for each of the cases above: (d) - amplitudes $A(\theta, t)$ of higher harmonics versus θ and $t\dot{\epsilon}$ (values in color), (e) $|u_k|^2(t)$ are the instantaneous Fourier spectra of the amplitudes $A(\theta, t)$ at various $t\dot{\epsilon}$ (values in color), (f) $D(t)$ versus $t\dot{\epsilon}$. Columns ex_a, ex_b, ex_c correspond to the data presented in row (a), (b), (c).

in direction of the velocity gradient, $\dot{\epsilon}(t) = sign(t)\dot{\epsilon}_0$, is effectively equivalent to a negative surface tension and leads to the excitation of modes with $k^2 \leq \beta\chi/\sqrt{\Delta}$, where β is the numerical factor [17]. Then the theory gives $\chi_c \simeq 1.2$ for $k = 3$ and $\Delta \simeq 0.6$, which corresponds to our average $\langle \Delta_i \rangle$ over the data set in the transient region. The theoretical value of χ_c is of the same order as the experimental one, and the difference can be attributed, first, to uncertainty in the value of κ taken and to rather rough estimates based on an isotropic surface tension [17].

Another interesting phenomenon observed is the formation of buds. These could be seen intermittently in the experiments: sometimes the vesicle surface folds to

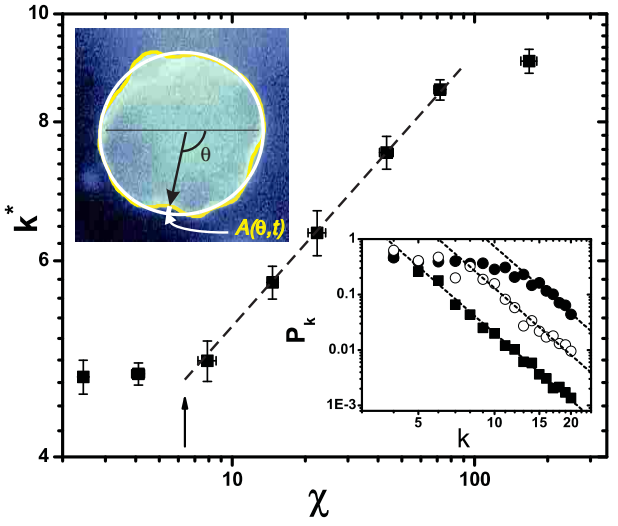

FIG. 3: k^* versus χ. The arrow defines χ_c, the onset of excitation of the mode $k = 3$. Dashed line is a fit $\sim \chi^{1/4}$ above the instability threshold. The upper inset illustrates the image analysis. Lower inset: averaged power spectrum for various χ: squares – 2.6; open circles – 24; circles – 116; dashed lines show a $\propto k^{-4}$ dependence.

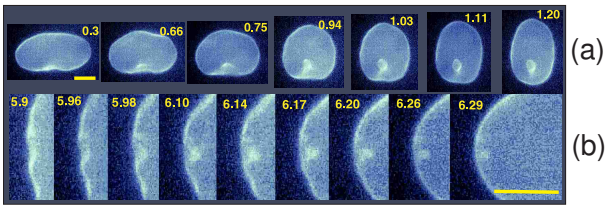

FIG. 4: Formation of buds. (a) $\chi = 6.8$, $\Delta = 0.9$; (b) $\chi = 179$, $\Delta = 0.4$. The scale bar is 10 μm, numbers are $t\dot{\epsilon}$.

the point that it creates the enclosure of a smaller vesicle inside the main one (Fig.4). We have not studied this phenomenon in detail, but some of its features can be described as follow: the enclosure process is irreversible, the bud scale is much smaller than the scale of the excited mode corresponding to the given χ, and the phenomenon is mostly seen in the vicinity of χ_c.

To summarize, we presented new experimental results about the relaxation dynamics of vesicles in elongation flows suddenly switched on or reversed. When the vesicle relaxes from its equilibrium shape towards a new stationary, stretched shape in the elongation flow, the scaling of the dynamics with the elongation rate and the linear dependence of the relaxation velocity on the viscosity contrast were found to be in agreement with the theoretical predictions [5, 6, 17]. We also observed and characterized a new instability, that results in the excitation of higher order modes, i.e. wrinkles, in the membrane, during the vesicle relaxation following the reversal of the velocity gradient. This unexpected generation of higher order modes suggests that only the appearance of a negative surface tension during the vesicle deflation due to compression in the transient can explain the effect. A recent theory [17] used this physical picture to derive a criterion for the onset of the instability and the power law dependence of the average wave number of the higher order modes as a function of χ, which is in reasonable agreement with our experiment. Finally we observed and report here, albeit without a quantitative investigation, the phenomenon of bud formation during the transient dynamics, particularly close to the wrinkling instability threshold.

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